

In the claims:

Please cancel, without prejudice, claims 23, 26, 45, 46, 65, 74-76, 84.

1. **(Currently amended)** An assay for identifying compounds that mimic a the bioactivity of a *hedgehog* protein, comprising:
  - (a) providing a cell that transduces intracellular signals of the hedgehog pathway and that expresses forming a reaction mixture including: (i) a naturally occurring *patched protein receptor*; and
  - (bii) contacting the cell with a test compound; and
  - (ciii) detecting activation of the hedgehog pathway in the cell by detecting a change in GLI expression in the cell, which GLI expression is responsive to the hedgehog pathway; wherein a statistically significant change in the activation of the *hedgehog* pathway in the presence of the test compound, relative to the activation in the absence of the test compound, indicates a *hedgehog*-mimicking activity for the test compound.

2. **(Cancelled)**

3. **(Currently amended)** The assay of claim 1, wherein the reaction mixture comprises a cell including a heterologous nucleic acid recombinantly expressing the *patched protein receptor*.

4. **(Withdrawn)** The assay of claim 1, wherein detecting activation of the *hedgehog* pathway comprises observing a phenotype of a cell in the presence and absence of the test compound.

5. **(Withdrawn)** The assay of claim 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of an intracellular second messenger responsive to signaling by the *patched* polypeptide.

6. **(Currently amended)** The assay of claim 17 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of expression of a gene controlled

by a *GLI* transcriptional regulatory sequence element, which *GLI* transcriptional regulatory element is responsive to the hedgehog pathway signaling by the *patched* polypeptide.

7. (Currently amended) The assay of claim 3, wherein the recombinant cell substantially lacks expression of an endogenous *patched* protein receptor.

8-16. (Cancelled)

17. (Currently amended) An assay for screening test compounds to identify agents that mimic a bioactivity of a *hedgehog* protein, comprising:

- i(a). providing a cell expressing a naturally occurring *patched* protein, which cell transduces intracellular signals of the hedgehog pathway *hedgehog* receptor, wherein said *patched* protein *hedgehog* receptor binds a naturally occurring *hedgehog* protein polypeptide;
- ii(b). contacting the cell with a test compound; and
- iii(c). detecting activation of the *hedgehog* pathway in the cell by detecting a change in the level of expression of a gene controlled by a *GLI* transcriptional regulatory element, which *GLI* transcriptional regulatory element is responsive to the hedgehog pathway, wherein a statistically significant change in the level of activation of the *hedgehog* pathway is indicative of an agent that mimics a bioactivity of a *hedgehog* protein.

18. (Withdrawn) The assay of claim 17, wherein the activation of the *hedgehog* pathway is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

19. (Withdrawn) The assay of claim 18, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

20. (Currently amended) The assay of claim 17, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a *GLI* transcriptional regulatory element sequence sensitive to intracellular signals transduced by activation of the

*hedgehog* pathway, and wherein expression of the reporter gene provides a signal for detecting activation of the *hedgehog* pathway.

21. **(Currently amended)** The assay of claim 20 or 33, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, or and drug resistance.

22. **(Currently amended)** The assay of claim 21, wherein the reporter gene encodes a gene product selected from chloramphenicol acetyl transferase, luciferase, betagalactosidase, or and alkaline phosphatase.

23. **(Cancelled)**

24. **(Withdrawn)** The assay of claim 17, wherein activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to activation of the hedgehog pathway.

25. **(Withdrawn)** The assay of claim 24, wherein the activation of the *hedgehog* pathway is detected by changes in intracellular protein phosphorylation.

26. **(Cancelled)**

27. **(Currently amended)** The assay of claim 17 or 26, wherein the cell further comprises a heterologous gene construct encoding the patched protein receptor.

28. **(Cancelled)**

29. **(Currently amended)** The assay of claim 17, wherein the cell further comprises one or more heterologous gene constructs encoding and expressing *costal-2*, *fused* and/or *smoothened* genes, or homologs thereof.

30. **(Currently amended)** An assay for screening test compounds to identify agents that activate the hedgehog pathway hedgehog signal transduction, comprising:
- i(a). providing a cell having a recombinant expression vector encoding a naturally occurring mammalian *patched* protein, wherein said cell transduces intracellular signals of the hedgehog pathway;
  - ii(b). contacting the cell with a test compound under conditions wherein the *patched* protein is expressed; and
  - iii(c). detecting a change in the hedgehog pathway in the cell by detecting a change in GLI expression in the cell or by detecting a change in the level of expression of a gene controlled by a GLI transcriptional regulatory element, which GLI transcriptional regulatory element is responsive to the hedgehog pathway signal transduction, wherein a ~~statistically significant~~ change in the hedgehog pathway signal transduction in the presence of the test compound, relative to in the absence of the test compound, is indicative of an agent that activates the hedgehog pathway signal transduction.
31. **(Withdrawn)** The assay of claim 30, wherein the signal transduction is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.
32. **(Previously presented)** The assay of claim 30, wherein the cell is a human cell.
33. **(Currently amended)** The assay of claim 30, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a GLI transcriptional regulatory element sequence sensitive to intracellular signals transduced by the hedgehog pathway signal transduction, and wherein expression of the reporter gene provides a detectable signal for detecting change in the hedgehog pathway signal transduction.
34. **(Currently amended)** The assay of claim 1,~~15, 26~~ or 30, wherein the *patched* protein is of vertebrate origin.

35. (Previously presented) The assay of claim 34, wherein the *patched* protein is a mammalian *patched* protein.
36. (Previously presented) The assay of claim 35, wherein the *patched* protein is a human *patched* protein.
- 37-41. (Cancelled)
42. (Previously presented) The assay of any of claims 3, 17, 30, or 78, wherein the cell is a metazoan cell.
43. (Previously presented) The assay of claim 42, wherein the cell is a mammalian cell.
44. (Previously presented) The assay of claim 42, wherein the cell is an insect cell.
- 45-46. (Cancelled).
47. (Currently amended) The assay of claim 1, 8,-17, 30, or 78, wherein the steps of the assay are repeated to screen a for a variegated library of at least 100 different test compounds.
48. (Currently amended) The assay of claims 1, 8,-17, 30, or 78, wherein the test compound is selected from small organic molecules or and natural product extracts.
49. (Currently amended) The assay of claim 1, 8,-17, 30, or 78, further comprising preparing a pharmaceutical preparation by combining of one or more compounds identified with a biologically acceptable medium.
- 50-62. (Cancelled)
63. (Currently amended) A method for identifying *hedgehog* agonists, comprising:

contacting a test agent with cells expressing a naturally occurring *patched* protein, which cells transduce intracellular signals of the hedgehog pathway and wherein said cells undergo a detectable response when contacted with a naturally occurring *hedgehog* protein; and comparing the response of said cells to the test agent with the response of similar cells to a naturally occurring *hedgehog* protein, which detectable response is a change in *GLI* expression in the cell or a change in the level of expression of a gene controlled by a *GLI* transcriptional regulatory element responsive to the hedgehog pathway, wherein induction of a response in the presence of the test agent similar to the response induced in the presence of the *hedgehog* protein is indicative of agonist activity of the test agent.

64. (Currently amended) A method of claim 63, wherein said detectable response comprises expression of a gene controlled by a *GLI* transcriptional regulatory element sequence responsive to *patched*-mediated *hedgehog* signaling.

65. (Cancelled)

66. (Previously presented) A method of claim 63, wherein said cells are transfected to express a recombinant form of the *patched* protein.

67. (Previously presented) A method of claim 63 or 66, wherein said cells are eukaryotic.

68. (Currently amended) A method of claim 63, 66, or 67, wherein said cells are vertebrate cells.

69. (Currently amended) A method of claim 63, 66, or 67, wherein said cells are mammalian cells.

70. (Currently amended) A method of claim 63, wherein said cells further comprise a reporter gene construct operably linked to a *GLI* transcriptional regulatory element responsive to *hedgehog* signaling, and said detectable response comprises detecting the level of expression of said reporter gene, and comparing the response of said cells to a test agent.

71. (Currently amended) A method of claim 70, wherein expression of the reporter gene is detected by detecting determining the protein product encoded by the reporter gene.

72. (Currently amended) A method of claim 71, wherein the reporter gene product is detected by measuring an intrinsic activity of the associated with that product.

73. (Currently amended) A method of claim 71, wherein the reporter gene product is detected by assaying for an enzymatic activity associated with that product.

74-77. (Cancelled).

78. (Currently amended) An assay for screening test compounds to identify agents that antagonize a bioactivity of a *hedgehog* protein, comprising:

- i(a). providing a cell expressing a naturally occurring *patched* protein, which cell transduces intracellular signals of the hedgehog pathway and *hedgehog receptor*, wherein said *patched* protein *hedgehog receptor* binds a naturally occurring *hedgehog protein polypeptide*;
- ii(b). detecting activation of the hedgehog pathway in said cell by detecting a change in *GLI* expression in the cell or by detecting a change in the level of expression of a gene controlled by a *GLI* transcriptional regulatory element, which *GLI* transcriptional regulatory element is responsive to the hedgehog pathway;
- iii(c). contacting the cell with a test compound;
- iv(d). detecting activation of the *hedgehog* pathway in said cell in the presence of said test compound by detecting a change in *GLI* expression in the cell or by detecting a change in the level of expression of a gene controlled by a *GLI* transcriptional regulatory element, which *GLI* transcriptional regulatory element is responsive to the hedgehog pathway, wherein a statistically significant decrease in the level of activation of the *hedgehog* pathway in the presence of said test compound in comparison to the absence of said test compound is indicative of an agent that antagonizes a bioactivity of a *hedgehog* protein.

79. (Withdrawn) The assay of claim 78, wherein the activation of the *hedgehog* pathway is detected by detecting a change in phenotype of the cell in the presence of the test compound.

80. (Withdrawn) The assay of claim 79, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

81. (Currently amended) The assay of claim 78, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a *GLI* transcriptional regulatory element sequence sensitive to intracellular signals transduced by activation of the *hedgehog* pathway, and wherein expression of the reporter gene provides a signal for detecting activation of the *hedgehog* pathway.

82. (Currently amended) The assay of claim 81, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, or and drug resistance.

83. (Currently amended) The assay of claim 82, wherein the reporter gene encodes a gene product selected from chloramphenicol acetyl transferase, luciferase, betagalactosidase, or and alkaline phosphatase.

84. (Cancelled)

85. (Withdrawn) The assay of claim 78, wherein activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to activation of the hedgehog pathway.

86. (Withdrawn) The assay of claim 85, wherein the activation of the *hedgehog* pathway is detected by changes in intracellular protein phosphorylation.

87. (Withdrawn) A method of manufacturing an agent that activates hedgehog signal transduction, comprising

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. contacting the cell with a test compound;
- iii. detecting activation of *hedgehog* signal transduction,  
wherein a statistically significant change in the level of activation of *hedgehog* signal transduction is indicative of an agent that mimics a bioactivity of a *hedgehog* protein; and
- iv. synthesizing said compound so identified as an agent that mimics a bioactivity of a *hedgehog* protein.

88. (Withdrawn) A method of manufacturing an agent that antagonizes *hedgehog* signal transduction, comprising

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. detecting activation of *hedgehog* signal transduction in said cell;
- iii. contacting the cell with a test compound;
- iv. detecting activation of *hedgehog* signal transduction in said cell in the presence of said test compound,  
wherein a statistically significant decrease in the level of activation of *hedgehog* signal transduction in the presence of said test compound in comparison to the absence of said test compound is indicative of an agent that antagonizes *hedgehog* signal transduction; and
- iv. synthesizing said compound so identified as an agent that antagonizes *hedgehog* signal transduction.

89. (Withdrawn) The method of claim 87 or 88, further comprising formulating said agent in a pharmaceutically acceptable carrier.

90. (Withdrawn) An agent that antagonizes *hedgehog* signal transduction identified by the assay of claim 78.

91. (Withdrawn) An agent that activates hedgehog signal transduction identified by the assay of any of claims 17, 30 or 63.

Please add the following new claims:

92. (New) A method for identifying *hedgehog* antagonists, comprising:  
contacting a test compound with cells expressing a naturally occurring *patched* protein, which cells transduce intracellular signals of the hedgehog pathway and wherein said cells undergo a detectable response when contacted with a naturally occurring *hedgehog* protein; and  
comparing the response of said cells to the test compound with the response of similar cells to a naturally occurring *hedgehog* protein, which detectable response is a change in *GLI* expression in the cell or a change in the level of expression of a gene controlled by a *GLI* transcriptional regulatory element responsive to the hedgehog pathway, wherein a decrease in the detectable response in the presence of the test compound in comparison to the detectable response induced in the presence of the hedgehog protein indicates antagonist activity of the test compound.

93. (New) A method of claim 92, wherein said detectable response comprises expression of a gene controlled by a *GLI* transcriptional regulatory element responsive to *patched*-mediated *hedgehog* signaling.